

THE CIRCADIAN RHYTHMS OF HUMAN SUBJECTS WITHOUT TIMEPIECES OR INDICATION OF THE ALTERNATION OF DAY AND NIGHT

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SUMMARY

1. Seven solitary subjects, and two groups of four, spent from 5 to 13 days in an isolation unit without knowledge of time. Three solitary subjects and one group of four adopted fairly regular activity habits with a period of 25–27 h; one subject adopted a period of 30 h, and one of 27 h initially, decreasing to 24–25 h after a few days. One group of four awoke roughly every 24 h, after a sleep which was alternately about 8 h, or about 4 h and believed by the subjects to be an afternoon siesta. Two solitary subjects alternated sleeps of about 8 or 16 h, separated by 24 h of activity.

2. Deep temperature in all subjects oscillated with a period of 24–26 h, which was thus commonly distinct from their activity habits.

3. Urinary potassium followed a rhythm whose period, though usually close to, was sometimes distinct from, that of temperature. A secondary period corresponding to that of activity was also sometimes present.

4. Urinary sodium and chloride usually gave evidence of two periodic components, one corresponding to activity and the other to the rhythm of either temperature or of urinary potassium.

5. Urinary creatinine and phosphate usually followed the subject's routine of activity.

6. Plasma samples were collected on a few occasions and analysed for phosphate and 11-hydroxycorticosteroids. Changes in plasma phosphate were usually, but not always, associated with similar changes in urinary phosphate, and changes in plasma corticosteroids were often, but not always, associated with similar changes in urinary potassium shortly afterwards.

7. Observations are recorded on a subject alone in a cave for 127 days. His activity habits, though wildly variable, gave evidence of a period of 25.1 h and his urinary electrolyte excretion indicated a shorter period, of 24.6 h. During the following 3 days, when he remained in the cave but was

visited frequently, his plasma corticosteroids and urinary potassium oscillated with a period of 16 h.

8. The possible mechanisms controlling these rhythms are discussed.

Observations upon men, other animals, and plants isolated from such external periodic influences as the alternation of day and night are commonly described as 'free-running'. The usual supposition is that any rhythms observed under these circumstances must arise from within the organism, and such observations have been extensively used to demonstrate the existence of endogenous rhythms, and to elucidate their characteristics. More specifically, any dissociation between the behaviour of different rhythmic variables is used as evidence against a close causal connexion between them, and is often interpreted as suggesting the presence of two or more independent controlling oscillators. We report here a series of observations on human subjects deprived of knowledge of time in which a wide range of different variables has been simultaneously recorded.

It is commonly assumed that the pattern of sleep or rest and activity in free-running experiments reflects an internal rhythm of sleepiness and wakefulness; but though there is good evidence that such a rhythm exists, it may not be the only factor determining the time when a human subject in isolation wakes or retires to bed. On the other hand, the routine of rest and activity, with the resultant pattern of ingestion of food and drink, can at many points influence other physiological variables such as body temperature and renal excretion. A rhythm whose period is the same as that of sleep and wakefulness, and whose phase relationship is similar to that observed during customary nycthemeral existence, may therefore be determined by those habits, and not be endogenous. We have therefore paid particular attention to how far the observed rhythms have or have not conformed with the pattern of sleep and activity adopted by the subjects.

METHODS

Our isolation unit has been described elsewhere (Elliott, Mills, Minors & Waterhouse, 1972). Within this unit 15 subjects have spent 5–13 days without indication of the alternation of day and night unless, as Wever (1967, 1970, 1971) maintains, they could be influenced by the earth's magnetic field. They were all asked to follow a regular existence, rising, taking meals, and retiring at what seemed to them their customary times; the actual times were recorded by the subjects pressing appropriately coded keys which operated a time-coded print-out on paper strip outside the unit. We studied seven subjects, designated G1–7, alone, and two groups of four, designated H1–4 and J1–4. Further details are given in Table 1.

Some subjects spent a few days in the unit with a clock, either immediately before or after their 'timeless' sojourn. When the clock was started afterwards, it was

started at the time which the subject believed it to be, and the subject was not told the real time.

All subjects were healthy volunteer students, mainly medical but otherwise selected only by reason of availability, except that G1 was chosen for his unusual sleeping habits, and G5 is a physiologist working on salivary rhythms, which he recorded at the same time as the observations here described. We also include some data from a subject D.L. who spent 4 months alone in a deep cave; these have been briefly reported elsewhere (Mills, 1967*a*, 1967*b*; Conroy, 1967). He had no timepiece and therefore was 'free-running', but he had a telephone link to the surface, permanently manned, through which he indicated his times of retiring and rising. On nine occasions, starting at the time of rising, he collected separately every urine sample he passed for 22-48 h, comprising seven to twelve consecutive samples. He measured the volumes, telephoned to the surface to indicate the times, and kept an aliquot of each until the series was complete, when the samples were collected from a prearranged place while he slept and were sent to Manchester for analysis.

TABLE 1. Details of subjects studied

Subject	Sex	Age (yr)	Time spent without knowledge of time	Time spent in unit with clock
G1	M	22	5 d 3.5 h	4 d 13.5 h, after
G2	F	27	5 d 1.9 h	3 d 22.8 h, after
G3	M	21	13 d 1.1 h	—
G4	M	19	9 d 18.5 h	—
G5	M	35	12 d 23.7 h	2 d 10.8 h, before
G6	M	19	11 d 23.2 h	—
G7	M	19	7 d 18.9 h	—
H1, 3 and 4	M	19, 19, 20	9 d 18 h	—
H2	M	20	8 d 17.2 h	—
J1-4	F	18, 19, 18, 19	9 d 2 h	2 d 16 h, before
D.L.	M	28	127 days, in cave	—

After he had completed 127 days in solitude he was informed of this fact, but not of the time, and he consented to remain for some time further so that we could collect a series of blood samples. During the next 3 days he collected all his urine samples as before, and we visited him every 4 h or so to collect a blood sample. We did not tell him how frequently we were visiting him: he thought the intervals were about 2 h; and we took great care to avoid giving him any clue as to the alternation of day and night; we refrained from shaving during these days. His self-chosen times of sleep were of course disturbed by our frequent visits; but when we found him asleep we usually managed to descend into the cave and make preparations without waking him until we were ready to collect blood, and he was enabled to resume his sleep with a minimum of delay.

Methods of measurement of temperature, and of analysis of plasma and urine, were as described by Elliott *et al.* (1972), except that some subjects measured temperature rectally, and in G7 recording was continued by telemetry from a rectal probe while he slept. A few of the later urine analyses were performed by Technicon AutoAnalyzer.

When temperature was measured both orally and in urine, a subjective decision was made as to which, in the particular subject, was more reliable, and this one was thereafter used consistently for that subject. Both methods of measurement, however, usually yielded very similar results on spectral analysis of the rhythms.

The methods used to assess the presence of rhythms and to define the period and the acrophase, or time of the statistically defined maximum, are described in the Appendix.

RESULTS

For convenience, the best-fitting periods of the different rhythms of all subjects are tabulated in Table 2. In all, the fitted sine curve was, by the criterion of variance ratio, significant at the 5% level and nearly always at the 1% level. Since however a significant fit could often be achieved with a slightly different period, these estimates of period are not always very precise. The different rhythmic functions will be considered separately except for those upon D.L., which differ in many respects from the others and will therefore be considered together.

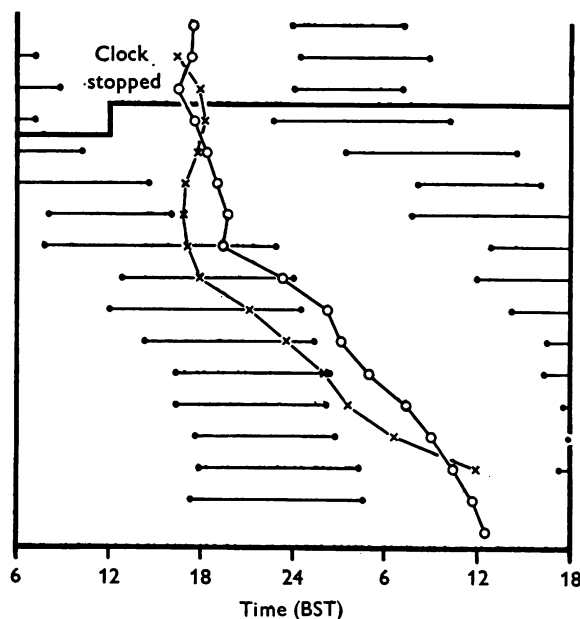


Fig. 1. Subject G5. Continuous bars represent time of sleep on successive days from above downwards, with the data for each day extended on the abscissa by 12 h. Acrophases of best-fitting sine curves of period 24 h are fitted to successive overlapping 72 h windows for temperature (○—○) and urinary sodium (×—×).

Sleep and activity patterns

Patterns of sleep and activity fell into two sharply distinct groups. Five of the solitary subjects, and one of the groups of four, group J, followed an activity cycle with a period slightly longer than 24 h, as have most subjects studied elsewhere. Two of the solitary subjects, G3 and G6, and the other

TABLE 2. Periods giving the best fit for different variables, derived as explained in Appendix. Values in hours

Subject	Temperature	Urinary					Activity	
		Flow	Potassium	Sodium	Chloride	Phosphate	Creatinine	<div>A B</div>
G1	U 25	22, 25, 30	26	25	26	25, 29	25	25.6 ± 0.2 —
G2	O 25	Nil	25	Nil	25	23	24	26.2 ± 0.03 25.9
G3	U 24, 37	24.4	24.1	24.8	24.4	24.1	Nil	36.8 ± 0.5 37.4
G4	U 25, 30	25	25, 30	25, 30	25	21, 30	25	30.0 ± 0.4 30.3
G5	{ U 25	23	23	24	24	24	23	27.4 ± 0.1 27.5
	{ U 26	28	27	27	27	25	28	24.7 ± 0.1 24.4
G6	R 25	25	25.0, 28.5	24.6	24.4	24, 21	23.4, 31	— —
G7	R 25	22	25, 21	26, 21	26, 21	24	25	25.7 ± 0.2 25.7
H1	O 25	24	24	24	24	31	25	23.8 ± 0.2 —
H2	U 24	—	—	—	—	—	—	23.7 ± 0.3 —
H3	U 24	24	24	24	24	24	23	23.7 ± 0.2 —
H4	U 26, 31	24	24	24	24	23	24	23.8 ± 0.2 —
J1	O 25	27	24	27, 23	28	27	26	26.8 ± 0.3 26.5
J2	O 24	27	24	28	24, 27	26	28	26.8 ± 0.3 26.4
J3	O 26	23	24	27	26	27	24	26.8 ± 0.3 26.6
J4	O 25	24	24	23, 27	24	26	Nil	26.8 ± 0.3 26.6

'Nil' indicates that no fitted sine curve was significant at the 5% level.

U, O and R indicate urinary, oral or rectal temperature; only in G7 was temperature recorded during sleep.

A and B indicate activity rhythms derived respectively by a linear regression, and by fitting a rectangular model, as described in the Appendix. The value for G1 includes his time without and with the clock. The analysis for G5 is divided into the first 5 (activity and temperature) or 6 (urine) and the last 8 or 7 days, as explained in the text. The activity rhythm by method A is derived from successive mid-points of sleep except for Group H, for which the time of waking has been used.

group of four, followed a pattern in which the times of sleep were alternately long and short.

The best estimate of the recurring period of the regular activity cycles is shown in Table 2; some of these deserve further specific comment. The record of sleep in subject G5, as indicated in Fig. 1, suggests that for the first 5 days of isolation the period of his activity cycle was much longer than during the last 8 days, and separate estimates are therefore presented.

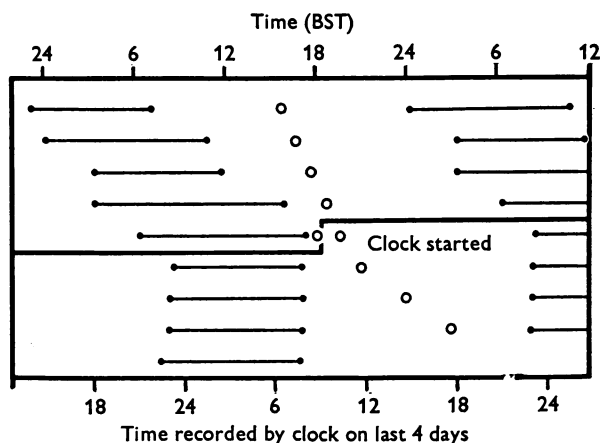


Fig. 2. Subject G2. Record of sleep, indicated as in Fig. 3 except that the abscissa is extended by 14 h. \circ , successive acrophases of sine curves fitted to the whole stretch of data for potassium excretion, before (25 h period) and after (27 h period) the clock was started. The last day without a clock is included in both analyses, so two acrophases are indicated on this day.

Subject G1, already briefly reported (Elliott, Mills & Waterhouse, 1971), was selected because he maintained that in ordinary life he had difficulty in living on a day as short as 24 h. Records of his times of rising and retiring while living in society tend to confirm this: he would retire and rise progressively later for a series of nights until some factor, described by himself as feeling able to sleep, eventually compelled him to have an early night and return to normal phasing. A histogram derived from 36 such days has a well defined mode at $24\frac{1}{2}$ – $25\frac{1}{2}$ h for the interval from one time of retiring to the next, with a smaller mode at $20\frac{1}{2}$ – $21\frac{1}{2}$ h, which presumably represents the days when he returned to normal time. In isolation for 5 days he followed an activity rhythm with a period around $25\frac{1}{2}$ h, which is in no way remarkable. The clock was then started, and he was asked to try to go to bed and rise at regular and conventional hours. He was informed that the clock could be adjusted to gain or lose as much as 3 h

in 24, and that he would not be told whether it was set to gain or lose. In fact, it was set to keep correct time, but he maintained that he was unable to conform with it, and followed an activity cycle of about the same period as when he had no knowledge of the time. A single estimate of the length of his activity cycle was therefore derived from his 10 days in the isolation unit, yielding a period of 25.6 ± 0.2 h.

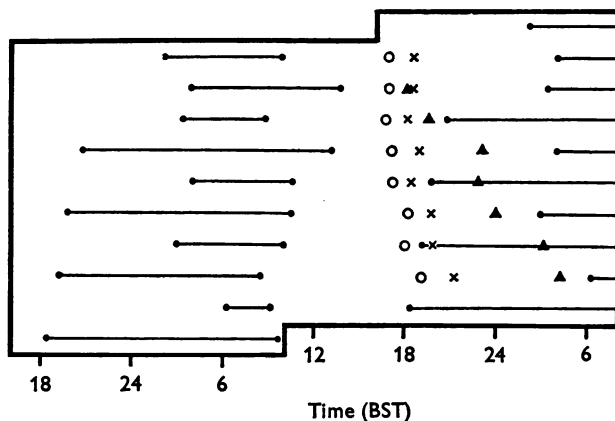


Fig. 3. Subject H4. Record of sleep, indicated as in Fig. 1, with abscissa extended by 16 h. Acrophases of sine curves fitted to successive 72 h windows for ▲, temperature (period 26 h), ○, potassium excretion (period 24 h) and ×, sodium excretion (period 24 h).

Fig. 2 shows, for contrast, another subject, G2, treated in the same way, who followed a similar rhythm when deprived of knowledge of the time, but had no difficulty in conforming to a 24 h period when the clock was started.

The spontaneity of the sleeping habits of group J (Fig. 5) was disturbed on their fourth and eighth nights after the clock was stopped, since on these nights they collected blood samples. A sample was collected immediately before retiring, and two of the subjects stayed up for about $\frac{3}{4}$ – $\frac{1}{2}$ h to centrifuge the blood and pipette off the plasma. All four were then awakened about halfway through their sleep to collect another sample and, presumably as a result, they slept late next morning. For analysis of activity rhythms these broken nights have been treated as if sleep were uninterrupted, as were also the occasional nights when other subjects were awakened by the need to micturate. All these four subjects of group J followed activity cycles with a period of 26.8 ± 0.3 h. Of the other two subjects who followed more or less regular habits, G7 adopted a shorter period of 25.7 ± 0.2 h, and G4 an unusually long period of 30.0 ± 0.4 h.

The sleeping times of H4, who was typical of his group, are shown in

Fig. 3. After the first 2 days they settled down to a routine in which sleeps of 14–16 h alternated with ones of 3–7 h. Their times of retiring were widely variable, but their times of waking were fairly regular and can be fitted by a period slightly, but not significantly, below 24 h, as indicated in Table 2. Their idea of time was totally erroneous, in that they thought that the shorter periods of sleep were after-lunch naps, and slept in their clothes in easy chairs, a mistake similar to that recorded by Siffre, Reinberg, Halberg, Ghata, Perdriel & Slind (1966) and by Fraisse, Siffre, Oleron & Zuili (1968).

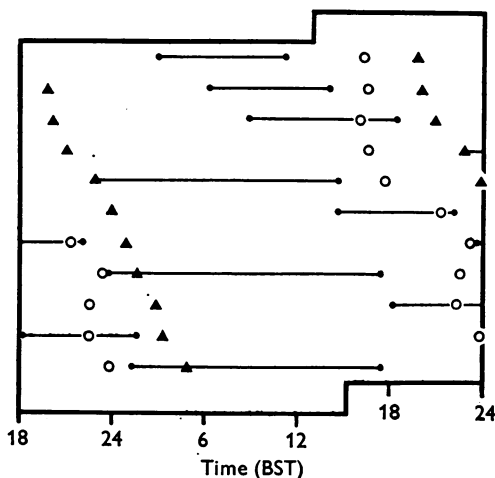


Fig. 4. Subject G6. Record of sleep, indicated as in Fig. 3 except that abscissa is extended by 6 h. Acrophases fitted to successive 72 h windows of rhythms of, ▲, temperature (period 25 h) and, ○, potassium excretion (period 25 h).

The behaviour of subjects G3 and G6 (Fig. 4) was so unusual that we were surprised to encounter it in two subjects. Their times of wakefulness lasted about 24 (23–28) h, but their sleep alternated regularly between long sleeps of 14–18 and short sleeps of 5–10 h. Times of retiring and rising were thus irregularly spaced, but mid-sleep recurred regularly about every 36 h; the period estimated for G3 from the rectangular fit was 37.4 h, and from linear regression 36.8 ± 0.5 h. Neither subject was aware of this behaviour; each supposed that he was retiring between 20.00 and midnight and rising between 08.00 and 09.00. In neither subject did this activity pattern emerge immediately in isolation. G3 spent 1, and G6 3, days initially on a fairly normal circadian routine before adopting the later pattern of long days and alternately long and short nights. The pattern in G6 cannot

therefore be objectively defined as well as in G3, although for the last 5 days it appeared similar.

Temperature

Despite the varied patterns of activity all subjects showed a clear-cut temperature rhythm with a period around or slightly longer than 24 h. For subjects G1, G2 and G7 the period could not be distinguished from that of the activity rhythm; for G4 it was substantially less; for group J it was less; but only in J2 was the difference sufficient to lead to a clear alteration of the usual phase relations, with the temperature acrophase drifting from about 16 h to only 6 h after mid-sleep.

In subject G5 (Fig. 1), as already described, the period of the activity rhythm appeared to change while he was in isolation. The temperature rhythm did not parallel this change, so that its acrophase drifted into the end of the sleep period after 5 or 6 days. By the end of the observations however it had returned to around the middle of the activity span. When his temperature acrophase fell during sleep the rhythm was of low amplitude and ill defined; but during the last 6 days it was again well defined and restored in amplitude, and appeared to have a period longer than that of the activity rhythm.

In subjects G3 and G6 (Fig. 4) the regular circadian rhythm of temperature contrasts with the highly irregular pattern of activity. In group H1, 2 and 3 the temperature rhythm of 24 or 25 h preserved the usual relationship with time of waking, but not with their irregular times of retiring, but in H4 (Fig. 3) the longer period, of 26 h, led to a grosser dissociation from his activity habits.

Secondary periods of 37 h in G3 and 30 h in G4 correspond well enough to their activity pattern, but a secondary period of 31 h in H4 admits of no such explanation.

Urinary constituents

When temperature and activity followed rhythms of similar period, the period of the rhythmic excretion of potassium, sodium and chloride was usually similar (subjects G1, G2, G7, H1, H3 and J3). In subject G2 (Fig. 2) the small difference between the periods of the potassium and activity rhythms led to a phase drift, whereby the acrophase occurred progressively earlier in the activity span. When the clock was started, the assumption of a 27 h period in the excretory rhythm brought the acrophase progressively later until after three days it had resumed its initial position in the activity cycle, around 17.00 by the clock. It should be noted however that this corresponds to 02.00 by solar time. Sodium and chloride excretion behaved similarly. In J3 (Fig. 5) the potassium rhythm was clearly distinct

from those of sodium, chloride, temperature and activity, having a period of 24 h. A secondary period of 21 h in all the electrolyte rhythms of G7 is likewise distinct from his other rhythms.

In most subjects in whom temperature and activity followed different rhythms (G3, G4, J1, J2, J4) potassium excretion followed a rhythm similar to that of temperature, while the excretory rhythms of sodium and/or chloride usually gave evidence of two components corresponding to the temperature and activity rhythms. Subject H4 (Fig. 3) was unusual in that potassium, sodium and chloride followed a rhythm with periods similar to one another and not very different from the pattern of activity, but

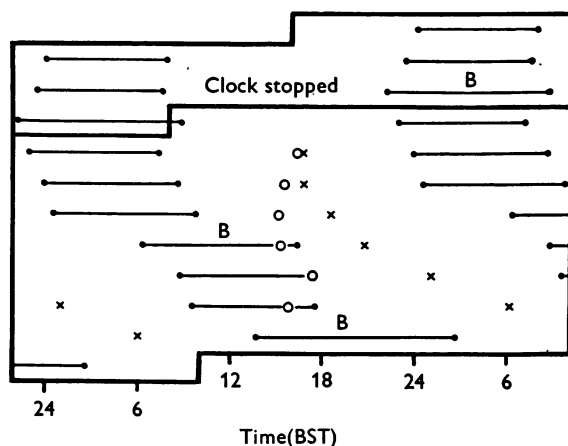


Fig. 5. Subject J3. Record of sleep, indicated as in Fig. 1. Acrophase of \times sodium and \circ potassium excretory rhythms, fitted to successive 72 h windows. B indicates nights when blood was collected.

notably shorter than the period of the temperature rhythm. In G6 (Fig. 4), in whom the activity was so irregular that no period could be assigned to it, the electrolyte rhythms were similar to those of temperature, and this is also true of G5 (Fig. 1), in whom the rhythms of electrolytes and temperature lengthened, and that of activity shortened, as his isolation continued.

Of other urinary constituents, excretion of creatinine seldom showed a well defined periodicity, but the best-fitting period was usually close to that of activity, reflecting the low rate of excretion during sleep. Occasionally however there was a circadian pattern in a subject whose activity pattern was not circadian, as in subject G4, whose activity period of 30 h does not appear in the analysis of creatinine excretion.

Excretion of phosphate also seemed to be largely determined by the habits, falling, as in a nycthemeral existence, when subjects rose from sleep, so that again the best-fitting period usually corresponded approxi-

mately to that of activity. A sine curve seldom provided a very good fit to the phosphate excretion; however in subjects G3 and G6, whose activity was irregular, excretion of phosphate had a circadian component; and in G4 there were two equally prominent periods, the one of 30 h corresponding to activity, and the other an unusually short circadian period of 21 h.

Since the volume of urine is known to be determined by many influences which may themselves vary circadianly (Mills, 1966) we have not paid much attention to analysing the rhythm of urine flow, which was usually similar to that of sodium and chloride. Only in one subject, G7, had the urine flow rhythm a period clearly distinct from the urinary electrolytes; his rhythm in urine flow did not match any of the other rhythms studied except the secondary period of his electrolyte excretion.

Plasma composition, urinary steroids

Of the solitary subjects only G1 collected blood samples, and he only collected them on retiring and on rising. The 11-OHCS concentration was consistently high on rising and low on retiring, even when his habits had shifted so far in relationship to solar time that he was rising shortly before midnight. Steroids were also estimated in his urine, and they bore a consistent relation to his potassium excretion, both being well fitted by a sine curve of period 26 h, with the acrophase of the urinary steroids about $\frac{1}{2}$ h later than that of potassium.

Subjects J2, 3 and 4 collected four to seven blood samples during each of three activity cycles, starting on rising and ending with a sample collected by interrupting their next sleep. This we facilitated either by visiting the subjects, or by providing them with 4 h timers to set on retiring. The days chosen were the last before the clock was stopped, and the fourth and seventh, by their reckoning, when they were free-running. The pattern of plasma 11-OHCS concentration was so similar in all three subjects that they are presented together in Fig. 6. On all days, a high value on waking was followed by an abrupt fall, as if cortisol release were conforming to the activity habits. The minimum value and subsequent rise occurred, however, successively earlier in the activity cycle, as if the adrenals were also influenced by a cycle distinct from that of activity. The phasing of the adrenal rhythm could not however be distinguished from that of urinary potassium, whose period was shorter than that of the activity cycle.

Subject J1 had poor veins, so blood was not collected and 17-OHCS were determined in urine instead. The best-fitting sine curve has a period of 26 h, and for the first 3 days the acrophases were within an hour or two of the potassium acrophases. Later, the potassium excretion did not follow a sinusoidal course and the course of steroid excretion was roughly parallel.

Group H attempted to collect blood samples on a similar routine to

group J, starting on waking on their third and sixth day of isolation, and ending with a sample on interrupting their next sleep. Owing to their grossly erroneous estimate of time they in fact started on the third and ninth day, continued through two activity spans and the intervening short

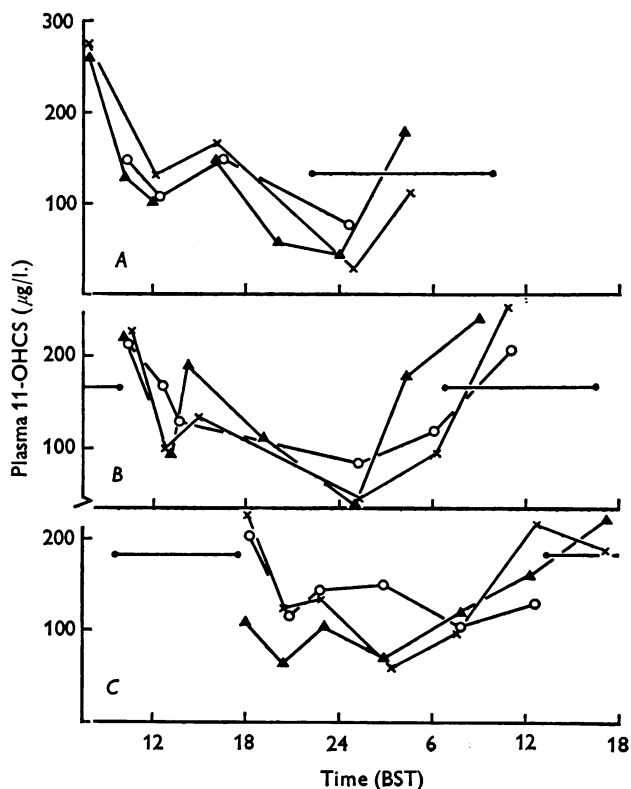


Fig. 6. Plasma 11-hydroxycorticosteroid concentrations, in subjects ▲ J2, × J3 and ○ J4. *A*, on third day in unit, with clock; *B*, on fourth day after clock stopped; *C*, on seventh day after clock stopped. Continuous bars indicate time in bed, ignoring interruptions for collection of blood.

sleep, which they interpreted as an after-lunch nap, and ended with a sample during the next long sleep; the five to seven samples collected on each occasion were thus spread over about 36 h. As with group J, the plasma 11-OHCS concentration was always high on waking and fell sharply thereafter; in this respect the supposed after-lunch naps behaved as normal sleep periods. The sampling was however too infrequent to permit any further definition of the time course.

The concentration of phosphate in the plasma varied much less than did

that of steroids. In subjects J3 and J4 it was usually high when they rose, fell thereafter for up to 8 h, and climbed again to high values in mid-sleep. In subject J2 the pattern was similar except for the absence of the early morning fall, suggesting that maximal concentration might have occurred late in sleep. On the seventh free-running day this pattern was modified in subject J2 and J3 by the addition of a marked peak during the activity span.

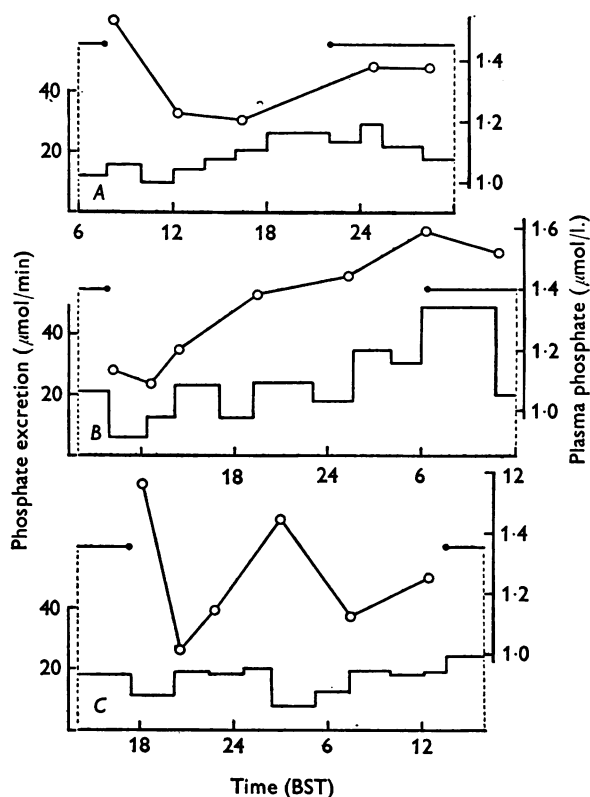


Fig. 7. Plasma phosphate concentration, and phosphate excretion. *A*, subject J3, second day in unit, with clock, *B*, subject J2, fourth day in unit without time; *C*, subject J4, seventh day in unit without time. Horizontal bars indicate time in bed.

In all three subjects on the control and fourth free-running days phosphate excretion changed roughly in parallel to the plasma concentration, but on the seventh free-running day this relationship no longer obtained. Examples are given in Fig. 7. To confirm this appearance, the correlation coefficients were calculated between plasma concentration and urinary excretion. The relationship appeared to be similar for all three subjects,

but different on the three days, so the variables for all three subjects were pooled, yielding correlation coefficients of 0.54 (14) for the control days and 0.63 (17) and -0.16 (18) for the fourth and seventh free-running days respectively; the parentheses indicate the number of degrees of freedom.

The rather infrequent voiding limits the correspondence that might be expected between plasma concentration and excretion rate, since the urine collection usually extended over 2 h after the plasma sample. In group H the much less frequent sampling of both plasma and urine, which resulted from their very inaccurate estimation of time, made it impossible to define these rhythms or any association between them.

Subject in isolation for 4 months

The routine of study in subject D.L., who spent 127 days in a cave in complete isolation, was in many respects different from that of the other subjects; his results are therefore considered separately. They have been briefly described elsewhere (Mills, 1967*a, b*, 1972; Conroy & Mills, 1970). The length of the 'day' adopted by this subject varied very widely, from 19 to 66 h, but when all the data are plotted in Fig. 8 a pattern emerges, in that the diagram is traversed by alternate dark and light bands running diagonally downwards to the right, indicating times when he was more likely to be asleep or awake. This suggests that his sleep and activity were determined by a rhythmic process with a period somewhat longer than 24 h.

The rectangular model for fitting sleep and activity gives a sharp optimum at 25.1 h, precisely the same as the period estimated by fitting a sine curve (Mills, 1967*b*, 1972; Conroy & Mills, 1970).

Urine was collected intermittently, for nine spans each covering 22-48 h and seven to twelve samples. In these 'windows' excretion of potassium, sodium and chloride often showed no obvious correspondence to the activity pattern, although on most occasions of sampling they appeared to be rhythmic. Sine curves have therefore been fitted to each individual 'window'.

Since the windows were too brief to permit definition of the period of any rhythm, a 24 h period was chosen and the fitted sine curves were used to estimate the acrophases of sodium excretion. These have been inserted in Fig. 8, where it is seen that they drifted progressively later. The inserted line is calculated as a conventional linear regression upon the number of days in isolation, and indicates a drift of 0.58 ± 0.05 h per day, defining a urinary sodium rhythm with a period whose 99% confidence limits are 24.39 to 24.77 h, shorter than the period of the activity rhythm of 25.1 h. The steady drift in relation both to clock time and to the activity habits is clear.

A similar regression of the chloride acrophases gave an indistinguishable period, of 24.63 ± 0.04 h, but the values fell about 0.6 h earlier.

In only 6 of the 9 'windows' could the urinary potassium be fitted by a sine curve, so its period cannot be calculated with the same precision; the 99 % confidence limits are 24.27 to 25.12 h.

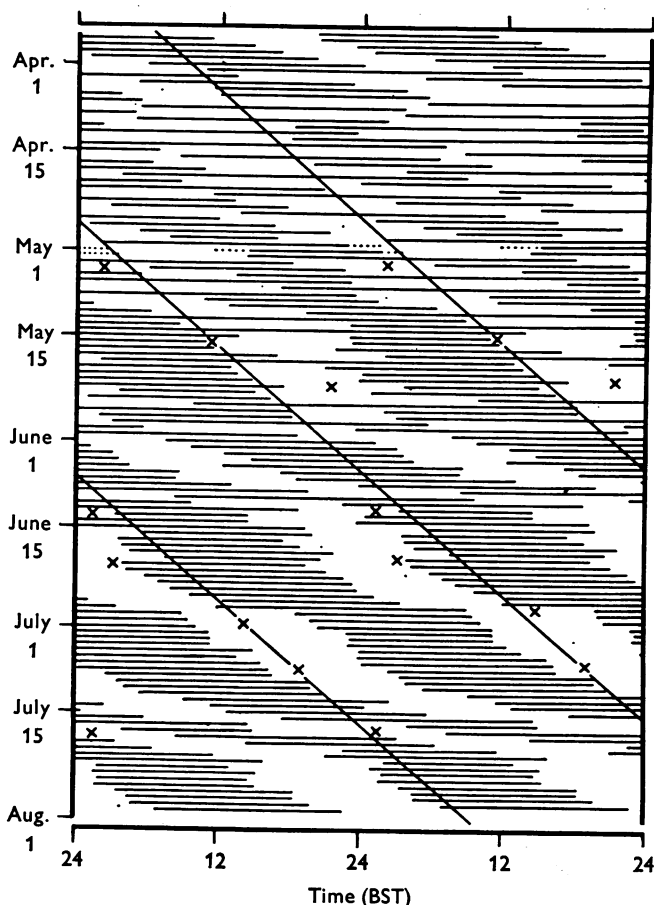


Fig. 8. Subject D.L. Record of sleep, indicated as in Fig. 1 except that the abscissa is extended by 24 h, so that all data are duplicated. x Acrophases of rhythm of sodium excretion, with linear regression upon number of days in cave. One window, where no significant sine curve could be fitted, has been omitted.

During the final 3 days, when all urine was kept and we visited the subject regularly to collect blood, sodium and chloride excretion were irregular. Potassium excretion followed a pattern which was neither circadian nor in conformity with the activity habits (Fig. 9), but best fitted by a

period of 16 h. As has been reported elsewhere (Conroy, 1967), the plasma steroid concentration during these 3 days also oscillated with a period of about 16 h.

Since it has been suggested that the cortisol rhythm may be responsible for the rhythm in potassium excretion, an attempt was made to correlate these two variables. The delay between the entrance of cortisol into the

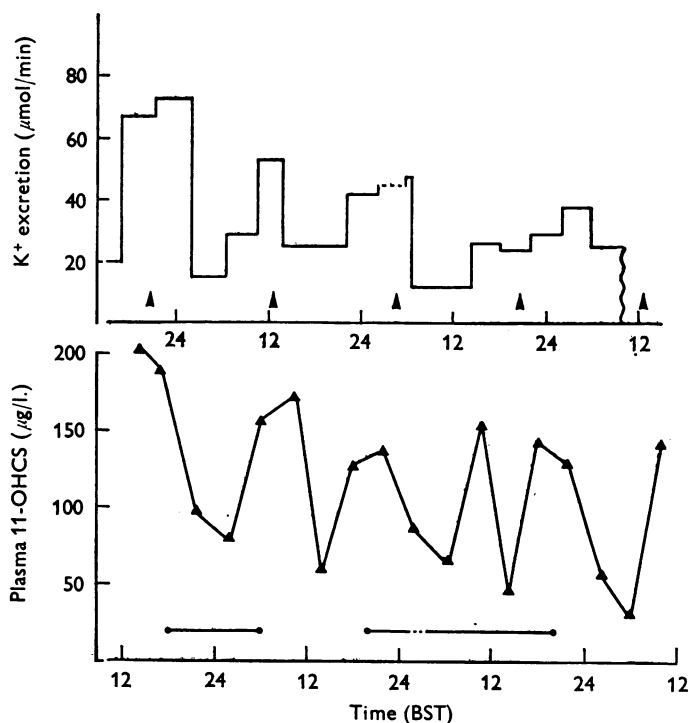


Fig. 9. Subject D.L., final 3 days after 4 months alone in cave. Above, potassium excretion; arrows indicate acrophases of fitted sine curve. Below, plasma 11-hydroxycorticosteroid concentration. Continuous lines indicate sleep, which was interrupted when blood samples were taken. The lower time scale is shifted by 5 h to show the approximate correspondence between excretory peaks and steroid peaks 5 h earlier.

plasma and its action upon renal excretion is not precisely known, so different delays were tested. The correlation coefficient between plasma cortisol concentration and potassium excretion 5 h later was 0.657, a highly significant value ($P < 0.01$). In Fig. 9 potassium excretion has therefore been plotted on a time scale 5 h behind that of plasma steroid, to illustrate this correlation, and the acrophases of excretion predicted by a sine curve with period 16 h are indicated.

Throughout the 4 months of isolation, excretion of phosphate and creatinine conformed very closely to the usual pattern of dependence upon sleep and activity. On every occasion when urine was collected, the phosphate excretion was in the first waking sample below the rate during sleep, in the second waking sample lower still, and on most occasions still lower in the third waking sample. The mean creatinine excretion rate during sleep was below that in the adjacent waking time on eight out of nine occasions; the (geometrical) mean of the ratio of waking to sleeping excretion rate was 1.26.

DISCUSSION

Observations upon organisms deprived of any nycthemeral clues have a twofold interest; the occurrence of rhythmicity in the absence of external rhythmic events gives evidence of rhythmicity inherent in the organism; and any dissociation between different rhythms implies a lack of any close causal connexion between them.

Other workers have recorded observations upon human subjects thus isolated from time information, either in underground bunkers (Aschoff & Wever, 1962; Aschoff, Gerecke & Wever, 1967*a, b*; Aschoff, 1969) or in deep caves underground (Siffre, 1963, 1965; Halberg, Siffre, Engeli, Hillman & Reinberg, 1965; Siffre *et al.* 1966; Fraisse *et al.* 1968; Colin, Timbal, Boutelier, Houdas & Siffre, 1969; Apfelbaum, Reinberg, Nillus & Halberg, 1969; Ghata, Halberg, Reinberg & Siffre, 1969; Reinberg, 1971). All are agreed that the spontaneously adopted pattern of sleep and activity follows a cycle with a period slightly in excess of 24 h, which is generally attributed to an endogenous timing mechanism. There is ample evidence, discussed by Conroy & Mills (1970, Ch. 7) that such a mechanism exists; it is perhaps most dramatic in subjects continuously awake (Fröberg, Karlsson, Levi & Lidberg, 1972), and it is very generally held responsible for the observed behaviour of subjects in isolation.

Aschoff, Gerecke, Kureck, Pohl, Rieger, von Saint Paul & Wever (1971) have argued in more detail that activity habits are ascribable to an endogenous oscillator, 'model B', which twice within each cycle crosses the threshold which determines whether the subject shall be awake or asleep, and thus determines the time both of retiring and rising. If the threshold alters, the time spent asleep may diminish and the time awake increase, but the period of the rhythm will be unaltered. As well as accounting for any regular alternation of sleep and activity this model could account for the behaviour of subjects G3 and G6, if the period of the oscillator were as long as 37 h and the threshold itself variable. It could not account for the behaviour of Group H, which is better fitted by a different model, 'A', rejected by these workers: one in which the oscillator determines the time

of arousal from sleep, but the time of retiring to bed is dependent not upon the behaviour of the oscillator but upon some other process such as fatigue.

Occasional or repeated activity cycles of around twice the usual circadian duration have been recorded by Aschoff *et al.* (1967*a, b*), and by Colin *et al.* (1968) as well as by ourselves (Mills, 1967*a*). These can be more readily explained by a slight modification of model A: if a subject retires at the time of maximal sleepiness as recorded by the oscillator, then if for any reason, such as engrossing activity, he did not go to bed at the moment of maximal sleepiness, he might feel no desire to do so until the next maximally sleepy moment, some 24 h later. The clear existence of a period of about 25 h in the rather irregular data of D.L. similarly suggests the regular recurrence of moments when he was most likely to retire or to rise.

We can accept intuitively that there are causes other than regularly alternating sleepiness and wakefulness which determine our hours of retiring, and perhaps also of rising, factors such as interest in or boredom with what we are doing, and also perhaps anticipation of what we are to do next day. We may also accept that fatigue sufficient to impel one to retire will inevitably supervene after a sufficient duration of wakefulness.

There is also a social, group influence; Apfelbaum *et al.* (1969) and Reinberg (1971) describe the activity habits of two groups of subjects who were sleeping in different tents but in the same cave; those in the same tent behaved similarly to one another, but differently from those in the other tent. Our groups of four subjects living together were asked to try to rise and retire at about the same time as one another; it is therefore according to expectation that an idiosyncratic behaviour, such as that of group H, should be common to all four members of the group.

It is possible that the endogenous influence is much stronger in some subjects than in others in whom different factors largely determine their habits. The cause of the peculiar behaviour of group H and of subjects G3 and G6 must be largely speculative, and need not be ascribed to an endogenous oscillator; it could be that a short night led to early fatigue and a long sleep on the following night, which left subjects so refreshed that on the next night a short sleep sufficed. Another explanation of the habits of group H would be that, since every alternate sleep was interpreted by them as a nap after lunch, they did not undress nor turn out the light, and consequently slept neither as deeply nor as long as during their longer sleeps when they undressed and went to bed in the usual way.

Whatever the explanation may be in individual instances, the times of retiring and rising of subjects living in isolation need not always be interpreted as indicating the operation of an endogenous mechanism which produces a rhythmic alternation of sleepiness and wakefulness. Consequently, dissociation between rhythms of activity and other rhythms may,

but does not necessarily, indicate the existence of two independent oscillators.

This variety in the sleeping habits of different subjects is however of particular value in studying the other components of physiological rhythmicity. When a subject in isolation follows an activity rhythm with a period of, say, 25 h, and his other rhythms follow a similar period, it is no more possible to investigate connexions between the different components than in subjects following an ordinary nycthemeral existence. Sleep, exercise, posture and meals are all potential influences upon temperature, endocrine secretion and renal function and any of them could well impart rhythmicity. The evidence for endogeneity is however much stronger when any component follows a rhythm clearly distinct from the activity pattern.

The variables studied on subjects isolated in caves have usually oscillated with a period similar to that of the sleep-activity rhythm, and such studies have therefore not helped much in elucidating causal relationships. Minor differences have however been observed in phasing. The two subjects studied by Ghata *et al.* (1969) showed an earlier phasing of their urinary 17-hydroxycorticosteroids in relation to sleep in isolation than when they were living outside, and in the male subject the temperature acrophase was only about 6 h instead of $14\frac{1}{2}$ h after mid-sleep. Aschoff *et al.* (1967*b*) observed a similar difference in the temperature acrophase in subjects in their bunker: when the light was continuous and there was no indication of time the temperature maximum fell in the first half of the activity span, but it fell in the latter half when subjects were exposed to a regular alternation of 12 h light and 12 h darkness. It is difficult to assess the importance of these minor differences in phasing, since they have not been subjected to the same close analysis as has the usual nycthemeral temperature oscillation. Further information comes from observations in space simulators (Rummel, Sallin & Lipscomb, 1967). Most workers have however recorded the rhythms in rather few physiological variables, so that observations upon their temporal relationships, from which inferences about causal connexions might be drawn, are limited.

Aschoff *et al.* (1967*a*) and Wever (1969), reviewing the results on subjects studied in isolation in their bunker, report that in most the observed rhythms were throughout synchronized with activity. Of more interest are subjects in whom for at least part of the time the period of the activity cycle was around 50 h, twice the period of the temperature cycle, and others in whom temperature and activity were for part of their stay entirely desynchronized, so that the temperature maximum changed continuously across the activity cycle. The commonest pattern among these nine was a temperature rhythm with a period somewhat over 24 h and an activity

period somewhat over 30 h, not very different from that of our subject G4. It is clear that in these subjects, as in our subjects G3, 5, 6, H4 and J2, and in subjects living on abnormal time schedules but retaining a circadian temperature rhythm (Mills, 1968), the temperature rhythm is endogenous and not determined solely by rest and activity. There is some interaction, however, for going to sleep at any hour is followed by a drop in temperature, so it is not surprising that the amplitude of the temperature rhythm may be less when it is out of phase with the pattern of activity (Aschoff *et al.* 1967*a*). This relationship emerges in subject G5, and the presence of two periods, one circadian and another corresponding to the activity rhythm, as in subjects G3 and G4, strongly suggests a secondary influence of sleep and activity.

Wever (1972) points out that subjects in isolation usually have a maximal temperature early in their activity span whereas, when they are living on a normal routine of day and night, temperature is usually highest much later in the activity span. The consequent shift of phase during the first few days in isolation will give the appearance that the period of the temperature rhythm is less than that of activity, an appearance which is belied when observations are prolonged. The potassium rhythm in subject G2 (Fig. 2) conformed precisely to this description. Subject G5 (Fig. 1) at first seemed to be behaving in this manner in an exaggerated form, in that his temperature maximum drifted right back into his sleep, but with more prolonged observation it shifted back into the normal position in the activity span. It is therefore uncertain whether in these two instances the behaviour should be described as a sudden change of period, or as a drift of phase.

In most subjects, the pattern of excretion of sodium, chloride and potassium followed rhythms with a period similar to one another, commonly also similar to that of temperature or activity, or even (G4, J2) giving evidence of two periods corresponding to the temperature and activity patterns. In subjects of group J, for example, whose activity rhythm had a period of 26.8 h, potassium excretion was best fitted by a period of 24 h, while sodium and chloride had optimal fits at 27 h. The dissociation in J3 (Fig. 5) was sufficient to bring the potassium acrophase into the sleep span. Similar dissociation has been observed by Elliott *et al.* (1972) in subjects shortly after a simulated time zone shift, with sodium excretion following the pattern of activity while potassium excretion was apparently determined by an endogenous rhythm.

The calculated period of the urinary sodium and chloride rhythms of 24.6 h for D.L. rests upon the assumption that the rhythm was consistent between the observed windows, which were separated by more than a week. The conclusion that the urinary rhythms were distinct from the

activity rhythm is therefore in this subject less secure, especially as sodium excretion was usually low during sleep.

Aschoff (1969) tentatively suggests that desynchronization is evidence for two independent endogenous oscillators, and Wever (1973) argue for a multiplicity of oscillators. This conclusion rests upon the assumption that, in these instances, the pattern of sleep and activity is determined by an endogenous oscillator; the limitations of this assumption have been already considered. Of much more evidential value is a dissociation between two rhythms neither of which is voluntarily determined, nor ascribable to the pattern of sleep and activity.

Accounts of such dissociation in the literature are very sparse. Rummel *et al.* (1967) describe dissociation between potassium excretion with a 30 h period, and sodium and 17-hydroxycorticosteroid excretion with a 24 h period, in a subject in a simulated spacecraft; the sleep and activity patterns are not described. We have found several more instances suggesting such dissociation.

In our subject H4, illustrated in Fig. 3, the urinary electrolyte rhythms have a much shorter period than the temperature rhythm, and the figure shows that neither rhythm corresponds closely to the pattern of waking and sleeping, even though the best-fitting period for the activity rhythm is close to that for urinary electrolytes. In J3 the 24 h period of the potassium rhythm is clearly distinct from the periods of sodium activity and temperature rhythms, as is the secondary 23 h period of the sodium rhythm in J1; and the secondary period of 24 h in the potassium, sodium and chloride rhythms of G7 is likewise distinct from the activity and temperature rhythms. In subject G5 it is the drift of the phase relations (Fig. 2) rather than a clearcut difference in period which suggests dissociation between electrolyte and temperature rhythms. In many subjects the rhythms were not definable with sufficient precision to detect such asynchrony, so we cannot say whether these dissociations are exceptional or usual.

The implications of such dissociations remain speculative, and have been discussed elsewhere (Mills, 1973). Since endogenous circadian rhythmicity has been observed in unicellular organisms, and in plant tissue culture, and animal tissues maintained *in vitro* (Bünning, 1967), there is nothing improbable about the supposition that such rhythmicity may reside independently in many human organs and tissues, although in the presence of the usual nycthemeral synchronizers all are constrained to the same period, as are the independently rhythmic tissues of the heart by the influence of the sinu-atrial node. Whatever the pathway of control between the 'master oscillator' and the rhythms we have observed, it seems that the control of temperature is causally distinct from that of urinary electrolytes. By contrast, most of the data are conformable with the suggestion

that a single circadianly varying influence controls excretion of the three electrolytes potassium, sodium and chloride, though they may also be affected by the habits of sleep and activity. Such possible exogenous influences include meals and changes in posture. When potassium is dissociated from sodium and chloride it may well be that these rhythms of habit are exerting a lesser influence upon potassium than upon sodium and chloride. If we accept that the temperature rhythm is mainly determined by an endogenous oscillator, even if its period is sometimes similar to that of the sleep and activity rhythm, then we have several examples which suggest the presence of two oscillators, one controlling temperature and the other electrolyte excretion. The evidence for any endogenous rhythmic influence upon excretion of phosphate and of creatinine is much slighter.

As has been pointed out already, consistent association between two or more rhythms is of less interest than is dissociation. The association between corticosteroid and urinary potassium rhythms deserves some attention, however, since cortisol is known to increase potassium excretion (Mills, Thomas & Williamson, 1960). The evidence for or against its responsibility for the circadian rhythm in potassium excretion, fully discussed by Mills (1973), is fairly evenly balanced. In the present experiments an association between potassium excretion and plasma or urinary steroids has been generally observed when both have been measured. The most interesting observations here are those during the last 3 days on subject D.L., when both plasma steroids and urinary potassium followed a rhythm with a period around 16 h. Little evidence is available on whether the phase delay of 5 h is plausible, if periodic steroid production were the cause of the rhythm in urinary potassium. Prolonged renal effects have been observed after single injections of cortisol (Mills & Thomas, 1958; Mills *et al.* 1960; Mills, Thomas & Williamson, 1961), but the doses used were larger than any likely to be secreted by the adrenals in a short period.

In the subjects of group J there was usually an association between a high level of plasma steroids and a rise in potassium excretion some hours later, though the time delay cannot be defined with any precision. Fig. 10A shows this relationship while the clock was running; the mid-day peak of potassium excretion after an early morning peak in plasma steroid concentration is a usual pattern, and in this instance a secondary plasma steroid peak in the afternoon precedes a secondary peak in potassium excretion. Fig. 10C shows the relationship in another subject on the seventh free-running day, when both steroid and potassium rhythms had drifted away from clock time; high steroid levels are still followed by high potassium excretion. A similar association was observed on the other days and subjects when blood was collected, except for the day illustrated in Fig. 10B; the high level of potassium excretion is here not preceded by a

steroid peak, nor is the high steroid level at 1729 followed by a rise in potassium excretion.

Since plasma steroid concentration is known to undergo quite large fluctuations over a period of an hour or two (Weitzman, Fukushima, Nogeire, Roffwarg, Gallagher & Hellman, 1971) it is impossible to define the pattern of plasma concentration precisely without very frequent sampling; and the infrequent sampling by group H, which resulted from

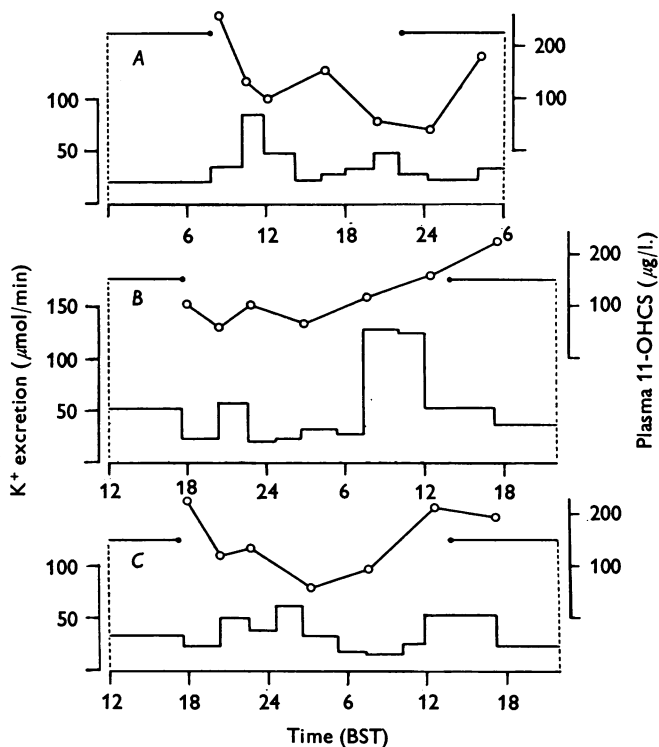


Fig. 10. Plasma 11-hydroxycorticosteroid concentration, and potassium excretion. *A*, subject J2, second day in unit, with clock. *B*, subject J2, and *C*, subject J3 seventh day in unit without time. Horizontal bars indicate sleep.

their false estimate of time, minimizes the usefulness of their plasma steroid values. It would seem however that cortisol secretion may be one, but certainly not the only, determinant of the potassium excretory rhythm.

The circadian rhythm in phosphate excretion is commonly paralleled by a rhythm in plasma phosphate concentration (Mills, 1966) and plasma concentration is known to be a major influence on urinary excretion (Anderson, 1955; Longson, Mills, Thomas & Yates, 1956), operating

through a Tm mechanism, though hormonal influences and varying glomerular filtration also affect excretion. The correlation we have observed in group J is probably as good as can be expected without more frequent sampling; but the lack of correlation on the seventh free-running day suggests that some other factor was now determining the phosphate excretory rhythm. Since creatinine excretion gives an approximate indication of glomerular filtration rate, the filtered load of phosphate should be proportional to the product of creatinine excretion and plasma phosphate concentration. We therefore also tried to correlate plasma phosphate concentration with the ratio of phosphate to creatinine excretion; the correlation was improved for the observations on the seventh free-running day, but was not significant at the 0.1 probability level. The immediate cause of the phosphate excretory rhythm is thus still obscure; but the evidence for an exogenous and a circadian component as well as the inadequate correlation with plasma concentration both point to a multiple causation.

We conclude that deep temperature and urinary electrolyte rhythms are controlled by independent timing mechanisms, distinct from one another and from the sleeping and activity habits. If activity is controlled by an endogenous rhythm this may also be distinct. Excretion of phosphate, creatinine, sodium and chloride, and sometimes potassium, are influenced also by the subject's habits; how far this influence results from changes of posture, from sleep, from meals, or from some other aspect of routine, is wholly speculative.

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APPENDIX

Statistical methods

Rhythms were sought by fitting by computer a cosine curve, using the least-squares criterion of best fit; for urinary data the deviant used was the difference between found and predicted amount of any excretory product in each collection period, as described by Fort & Mills (1970). Such a fitted cosine curve provides an estimate of the amplitude, A , and the time of the acrophase, or maximum predicted by the cosine curve, as well as the mean of the fitted cosine curve, termed 'mesor' by Halberg (1973); this can only fortuitously equal the mean of the actual observations unless the curve is fitted to an integral number of cycles, and the data are either continuously

sampled, as with urine, or uniformly spaced, which is unusual with temperature owing to the intervention of sleep.

Cosine curves were fitted with periods differing by 1 h, usually from 20 to 32 h but extending to a wider range if inspection of the data suggested the need; minimization of residual squared deviations, rather than amplitude, was used as the criterion of the best-fitting period. In a few instances a series of periods differing by 0.1 h was fitted, but our relatively short spans of observation did not appear to justify such precision in defining the period. A single period often stood out as providing a much better fit than those an hour shorter or longer, but with some series two distinct periods gave good fits, suggesting the operation of two distinct rhythmic influences with different periods.

Two periods may be present simultaneously in stationary data, or successively when the period changes during the course of observation. In stationary data two periods should interact to produce 'beats' with a period of $ab/[a \sim b]$, where a and b are the two periods present, as in the often-quoted result of Lewis & Lobban (1957, Figs. 2 and 3). In another very clear-cut example (Sasaki, 1972) a subject living on a 16 h day showed beats with a frequency of 48 h, as would result from interaction between the 16 h and a 24 h period. To search for beats, we fitted cosine curves to short spans of observation ('windows') of 72 h moving successively by 24 h across the whole span of observations – the 'pergressive analysis' of Halberg & Katinas (1973). When the amplitude of successive windows declined and then rose again with the appropriate beat frequency, we have accepted this as evidence for the simultaneous presence of two periods in the data. We have also accepted two periods as present when the secondary period both appears in the original analysis and also corresponds to a period present in another variable, usually in activity or temperature.

Such 72 h windows have usually been fitted with a fixed period of 24 h, since it is impossible to define the period from so short a window, and the computed amplitude and acrophase are hardly affected by moderate inaccuracy in the selected period. If the true period exceeds 24 h the acrophases of successive windows will fall progressively later, as in the temperature rhythms of Figs. 3 and 4. If the period of a rhythm changes during the observations, this drift of the acrophase will change its slope, as in temperature and sodium excretory rhythms of Fig. 1.

The objective specification of activity rhythms presents more difficulty, since neither sleep nor wakefulness can be quantified on a continuous scale. For moderately regular spans of observation we have used the simple technique of taking a definable point in the cycle, usually mid-sleep, and calculating the cumulative delay, in hours, after the time of mid-sleep in the first cycle. If, for example, successive times of mid-sleep were 3.0, 3.7,

4.5, 4.9... h after midnight, the calculated regression would be of these values upon the successive integers, 1, 2, 3, 4... representing the numbers of successive cycles. The linear regression coefficient of this cumulative delay upon the number of the cycle, when added to 24 h, provides an estimate of the period of the activity rhythm. The standard error of this regression coefficient is that of the estimated period only in so far as the time of sleeping is not consistently influenced by the time of sleeping on the previous 'night'.

We have also used another method. If wakefulness and sleep be given arbitrary values of 2 and 1, the best rhythmic model would be one in which identical spans of wakefulness alternate with sleeps which are all equal in duration to one another, though not necessarily equal to the spans of wakefulness. Deviations from this rectangular model can be expressed simply as the total time over which the model is faulty, since all deviations have the value of unity; and the period of the model with minimal deviation gives an estimate of the period of the activity rhythm. These two techniques give very similar values, as can be seen in Table 2. Only the latter method is however appropriate for subjects who show an occasional period of double the usual duration, as in Fig. 8.

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